

CONDUCTANCE OF THE SODIUM CHANNEL IN MYELINATED NERVE FIBRES WITH MODIFIED SODIUM INACTIVATION

BY F. CONTI,* B. HILLE,† B. NEUMCKE, W. NONNER‡ AND
R. STÄMPFLI

*From I. Physiologisches Institut der Universität des Saarlandes,
6650 Homburg/Saar, Federal Republic of Germany*

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SUMMARY

1. Na current fluctuations in nodes of Ranvier were measured under voltage clamp conditions as described in the preceding paper (Conti, Hille, Neumcke, Nonner & Stämpfli, 1976) and analysed in terms of power spectral density calculated for frequencies between 30 Hz and 5 kHz.

2. External (10^{-5} g/ml.) *Leiurus* scorpion venom or *Anemonia* Toxin II (3×10^{-5} g/ml.) or internal 20 mM iodate were applied in order to remove or slow down inactivation in part of the Na channels. The treatment increased the steady-state Na current during the noise measurement one- to eight fold over that in normal fibres.

3. Noise spectra were interpreted as the sum of $1/f$ noise and noise $S_{Na}(f)$ due to all-or-none, open-close transitions of single Na channels. The drug effects on the inactivation could be accounted for either by assuming two populations of channels, one with and one without inactivation, or by postulating a single population with modified inactivation characteristics.

4. Except for an increase in amplitude, the fluctuation spectra $S_{Na}(f)$ were similar to the ones in normal nodes. Again, the time constants τ_m obtained from the fit of the spectra agreed within a factor of 2 with the values of τ_m found in the macroscopic Na currents.

5. From the fluctuation spectra, single Na channel conductances γ of 5.4 ± 0.4 pS (iodate), 6.7 ± 0.5 pS (*Leiurus*) and 7.0 ± 0.6 pS (*Anemonia*) were calculated. The value of γ was not significantly voltage dependent.

6. Our observations indicate that inactivation of Na channels can be modified with at most small effects on the microscopic properties of the

* On leave from: Laboratorio di Cibernetica e Biofisica, CNR, Camogli 16032, Italy.

† Recipient of an award from the Alexander von Humboldt Foundation, Bonn-Bad Godesberg, Federal Republic of Germany and on leave from the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195, U.S.A.

‡ To whom reprint requests may be sent.

activation process and on the conductance of the open channel. They suggest that the *h* mechanism normally produces all-or-none, open-close changes of conductance.

INTRODUCTION

The previous paper describes the determination of the conductance of the Na channel from current fluctuations at the node of Ranvier (Conti *et al.* 1976). In the beginning of our work we reasoned that the experiments would be easier to do if the stationary Na current and the fluctuations were enhanced by blocking the process of Na inactivation. Without inactivation the theory for interpreting fluctuations would also be simpler. Results of such experiments are described in this paper using three agents which either block or delay Na inactivation. The experiments on fibres treated with iodate and scorpion venom were done before those in the previous paper and the experiments on fibres treated with *Anemonia* toxin were done at the end of the series. The major observations are that modification of inactivation has no major effect on single channel conductance or on the fluctuations of the *m*-gating process and the major conclusion is that the *h*-gating mechanism opens and closes in an all-or-nothing manner.

METHODS

The methods differed only in details from those given in the previous paper (Conti *et al.* 1976) as is described below.

Drug treatments. Fibres were treated with internal iodate by placing in pools *B*, *C* and *E* a solution containing 20 mM-KIO₃, 83 mM-KCl, 10 mM-CsCl, and 7 mM-NaCl. The ends of the fibre were cut in this solution and the iodate was allowed to diffuse into the fibre for 10 min before measurements were begun. The iodate solution remained in place throughout the experiment. External solutions in pool *A* were the standard test and TTX Ringer except in two experiments where a solution with all the NaCl replaced by tetramethylammonium (TMA) chloride was used instead of TTX Ringer. The external solutions were continuously flowing.

Fibres were treated with scorpion and *Anemonia* toxin by perfusing a few millilitres of toxin-containing test Ringer into the *A* pool then turning off the flow as only small quantities of toxin were available. After a few minutes measurements were begun. Later when power spectra in the test solution had been recorded, the flow was resumed and TTX Ringer without toxin was applied to permit recording corresponding power spectra with no Na current. Crude *Leiurus quinquestriatus* scorpion venom was obtained from Sigma Chemical Company. Purified *Anemonia sulcata* Toxin II (a peptide of molecular weight 4197) was a kind gift of Dr L. Beress, Kiel (Beress, Beress & Wunderer, 1975).

Recording of spectra. The precise sequence of amplifiers and filters and the type of low- and high-pass filters used differed from that in Fig. 1*C* of the previous paper (Conti *et al.* 1976), but in all pulse depolarizations the record was filtered at low and high frequencies of 22.3–63 Hz and 3.1–5 kHz. In the experiments with *Anemonia* toxin, which were done last, the high-pass filter was reduced to a simple one-pole circuit with blanking and the records were multiplied by a window function

suggested by Bingham, Dofrey & Tukey (1967) before taking the Fourier transform. For reasons given in the previous paper, both of these changes increase the reliability of low frequency spectral points.

Interpretation of spectra. In what way should modification of Na inactivation change the fluctuation spectra? Suppose that a drug treatment results in two populations of Na channels, one normal and the other modified. Designating the properties of these channels by subscripts A and B and using the same symbols as in the previous paper (i for current in an open channel and M for the number of channels), the total current is

$$I = I_A + I_B = m_A^3 h_A i_A M_A + m_B^3 h_B i_B M_B. \quad (1)$$

In these experiments we find that the drug treatments modify inactivation with at most small effects on m_∞ and τ_m , so some A and B designations can be dropped in the derivation. Finally if the h -gate of modified channels is held permanently open and if i_A equals i_B , the total current becomes

$$I = I_A + I_B = im^3(M_A h_A + M_B). \quad (2)$$

This two-population model has h noise from normal channels only and m noise from both. Generalizing from eqns. (19) and (20) of the previous paper and dropping subscript infinities as before gives for the spectral densities:

$$S_h(f) = \frac{4I_A^2}{M_A} \left(\frac{1-h_A}{h_A} \right) L(\tau_h, f), \quad (3)$$

$$\begin{aligned} S_m(f) &= 4 \left(\frac{I_A^2}{M_A h_A} + \frac{I_B^2}{M_B} \right) \sum_{b=1}^3 \binom{3}{b} \left(\frac{1-m}{m} \right)^b L(\tau_m/b, f) \\ &= \frac{4I^2}{M_A h_A + M_B} \sum_{b=1}^3 \binom{3}{b} \left(\frac{1-m}{m} \right)^b L(\tau_m/b, f), \end{aligned} \quad (4)$$

where $L(\tau, f)$ denotes a Lorentzian function of the form $\tau/[1 + (2\pi f \tau)^2]$. As would be expected, holding h -gates open increases I and S_m and decreases S_h , if the total number of channels $M_A + M_B$ remains constant.

The two population model was used to analyse power spectra measured on nodes treated with iodate or scorpion venom. The sum of eqns. (3) and (4) plus a $1/f$ component were fitted to the difference spectra

$$S_{diff} = \frac{N}{f} + S_{Na}(f) = \frac{N}{f} + S_h(f) + S_m(f) \quad (5)$$

taking as fixed parameters m , h_A , τ_h , and the ratio of modified to normal channels M_B/M_A and taking as variable parameters to be fitted τ_m and the amplitudes of the $1/f$ and the total $S_{Na}(f)$ components. The single channel current is then obtained by noting that $I/(M_A h + M_B)$ in eqn. (4) is equivalent to $m^3 i$. Making the substitution gives

$$i = \frac{S_m(0)}{4m^3 I \sum_{f=0}}, \quad (6)$$

where $S_m(0)$ and $\sum_{f=0}$ denote the zero-frequency values of $S_m(f)$ and the sum in eqn. (4). This equation is identical to eqn. (22) derived in the previous paper for a one-population model and contains no reference to A or B channel types. Therefore, errors in M_A/M_B have no effect on the calculated i provided that S_m is well fitted. It is not even important to know if there are two populations of Na channels instead of one. Errors in M_A/M_B would show up only in the low-frequency part of the spectrum as an under- or over-estimation of S_h . Since the relevant low-frequency

data points have very little statistical weight, the error would not bias the estimates of S_m significantly. With iodate and scorpion venom the fraction M_B/M_A was set at 1/3 to account for an average 75 % reduction of the peak Na current recorded at +60 mV when the prepulse was switched from -30 to +10 mV. Power spectra from nodes treated with *Anemonia* toxin were analysed by a one-population model using eqn. (25) in the previous paper. It should be emphasized that our experiments did not determine whether there are one or two channel populations after drug treatments and the choice between models was both arbitrary and unimportant for calculating channel conductances.

RESULTS

Modifications of the kinetics of Na current

The three pharmacological treatments used had obvious effects on the properties of sodium inactivation. Fig. 1*A* shows the time course of Na currents at thirteen different potentials in a normal nerve fibre under voltage clamp. Each pulse is preceded by a 100 msec hyperpolarizing prepulse. When the membrane is depolarized, Na current activates with time constants (τ_m) of 50–130 μ sec and then inactivates with time constants (τ_h) of 0.5–10 msec. When the membrane is repolarized, any remaining Na current turns off in less than 100 μ sec. Fig. 1*B* shows Na currents in the same fibre after a 7 min exposure to crude *Leiurus* venom, 10^{-5} g/ml. In agreement with the detailed description in the literature (Koppenhöfer & Schmidt, 1968*a, b*), the venom decreases the extent of inactivation developing during the pulse without much change of the fast activation or the fast turn-off of currents at the beginning and end of the pulse. The magnitude of the currents is also reduced in Fig. 1*B*, but this is primarily due to the absence of a hyperpolarizing prepulse rather than to an effect of the venom.

Internal iodate has effects similar to those of external *Leiurus* venom (Stämpfli, 1974). Fig. 1*C* and *D* show membrane currents in two different fibres 24 min and 41 min after the ends were cut in the solution containing 20 mM-KIO₃. A prepulse to +10 mV was used to observe the non-inactivating component of Na current. Although the pictures show that activation and turn-off of current are still fast, closer analysis reveals that τ_m and several other properties do actually change progressively as the iodate continues to act. In photographs taken after 9–14 min of action, τ_m and the m_∞ curve of the non-inactivating component of current are close to normal. Then gradually over the next 40 min, the peak current becomes smaller without much increase in the non-inactivating component, τ_m becomes larger, and the m_∞ curve becomes less steep. After perhaps 40–60 min the base-line current of the voltage clamp becomes unstable and the preparation is soon unusable. Except for this final decline, none of the gradual changes is dramatic. For example, in the

fibre of Fig. 1D, τ_m at 30 mV was 128 μ sec at 24 min. Our standard value of τ_m for a normal fibre is 121 μ sec at 30 mV. With very long depolarizations, what we have called the non-inactivating component declines

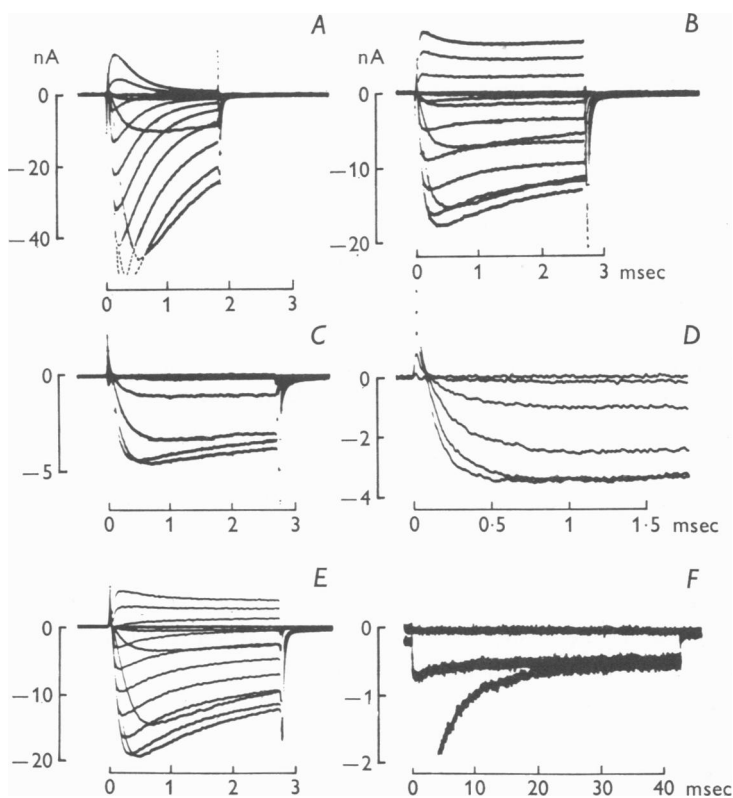


Fig. 1. Effects of drugs on macroscopic Na currents. *A*, node (45) in normal test Ringer; a 100 msec prepulse to -30 mV was followed by test pulses ranging from 10 to 130 mV in 10 mV steps. *B*, same node (45) after 7 min in test Ringer with *Leiurus* venom, 10^{-5} g/ml.; the same test steps as in *A* were applied without prepulse. *C*, node (40) in test Ringer, 24 min after cutting the fibre with the ends in axoplasm solution containing 20 mM iodate; 100 msec prepulses to $+10$ mV were followed by test pulses ranging from 10 to 60 mV in 10 mV steps. *D*, node (37) in test Ringer, at 41 min after cutting in the same solution as *C*; same pulses as in *C*. *E*, node (67) after 6 min in test Ringer with *Anemonia* Toxin II, 3×10^{-5} g/ml.; 100 msec prepulses to -30 mV were followed by test pulses ranging from 10 to 140 mV in 10 mV steps. *F*, same node (67) at 11 min after application of *Anemonia* Toxin II; a test pulse to $+30$ mV was preceded by a 100 msec prepulse to -20 mV (lowest trace) or $+20$ mV (middle trace), the current base line is marked by the upper trace. In *A*, *B*, *E*, and *F*, the capacity and leakage currents had been subtracted with the analogue circuit described in Methods of the preceding paper (Conti *et al.* 1976). K current was blocked by 10 mM-TEA in the test Ringer. Temperature 13° C.

with a time constant on the order of a minute. The same fibres will make single action potentials lasting longer than a minute in the TEA-containing Ringer.

The brief reports in the literature (Rathmayer *et al.* 1975; Romey & Lazdunski, 1975) suggest that *Anemonia* Toxin II reduces Na inactivation. Fig. 1*E* and *F* show sodium currents in a fibre treated with *Anemonia* Toxin II, 3×10^{-5} g/ml. for 6 and 11 min. The currents are quite similar to those in *Leiurus* venom (Fig. 1*B*) and have a non-inactivating component (Fig. 1*F*) that lasts longer than the *ca.* 250 msec time needed for fluctuation measurements. The activation parameters, τ_m and m_∞ , deduced from Fig. 1*E* are the same as those of a normal node, and the rundown of the fibres was not accelerated. The effect on inactivation cannot be described as a simple elimination of inactivation in certain channels because the inactivation curves showed a minimum in the potential range 30–60 mV, apparently quite analogous to that reported with *Leiurus* venom (Koppenhöfer & Schmidt, 1968*b*). This was seen as a slow increase of current (loss of inactivation) when the membrane was stepped to +90 mV from a prepulse to +40 mV. Action potentials in these fibres had a duration of 1 sec.

The traces in Fig. 1 were all from fibres used in fluctuation measurements. The drug doses chosen were in each case relatively mild and increased the steady inward Na currents by only one- to eight fold over that in normal fibres. Fluctuation measurements were begun 10–15 min after the beginning of the drug treatment.

Fluctuation measurements on modified fibres

The power density spectra of sodium current fluctuations were increased in amplitude by the drug treatments but were otherwise qualitatively similar to those from normal and Ni-treated fibres reported in the previous paper (Conti *et al.* 1976). Examples of spectra for iodate and *Leiurus*-treated fibres are given in Fig. 2 and, for *Anemonia*-treated fibres, in Fig. 3. The spectra in Fig. 2 were analysed by the two-population model given in the Methods assuming that 75% of the channels are normal and 25% have their *h*-gate held permanently open. The dashed curves show the S_m component from normal and modified channels (eqn. 4), the S_h component from normal channels (eqn. 3), and the $1/f$ component from all channels. On the other hand, the spectra in Fig. 3 were analysed by the one-population model of the previous paper. Standard values were used for τ_h , as was justified by the observed kinetics of voltage clamp current, and measured values of m_∞ and h_∞ were obtained from the fibres by conventional voltage clamp measurements. The measured spectra do not extend to low enough frequencies to decide the relative merits of the

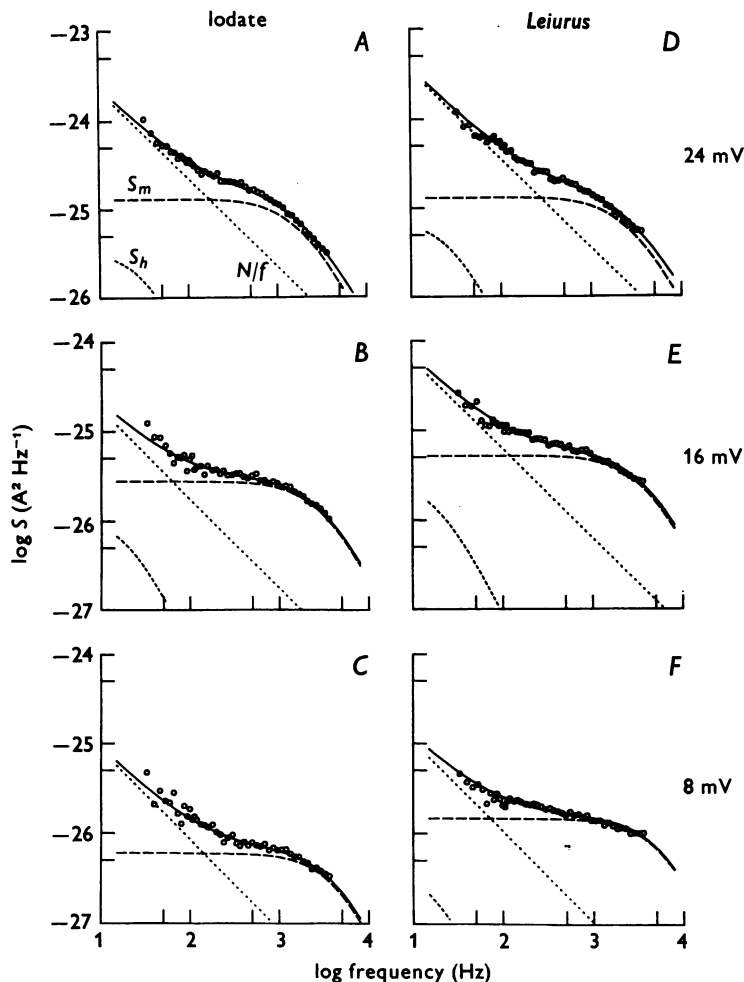


Fig. 2. Na current fluctuation spectra of drug-treated nodes. *A-C*, fibre (37) cut with the ends in axoplasm solution with 20 mM iodate. The node was first bathed in Na-free TMA Ringer and spectra for three potentials were measured between 11 and 18 min after cutting the fibre. Then, standard test Ringer was applied and spectra for the same potentials were taken between 23 and 38 min after cutting. The difference spectra for both solutions are shown. *D-F*, the node (43) was treated with *Leiurus* scorpion venom, 10^{-5} g/ml. by perfusing 3 ml. toxin-containing test Ringer through pool A. The flow was turned off and spectra at three potentials were measured at 7–19 min after application of toxin. Then, the perfusion was continued with TTX Ringer and the spectra were measured again at 30–39 min after toxin application. The difference spectra for both solutions were plotted. The continuous line was fitted according to eqn. (5). For comparison, the spectral components N/f , S_h and S_m are represented with dashed lines as marked in *A*. In *C*, S_h was off-scale.

one-population *vs.* the two-population model. However as is explained in the Methods, the model chosen has no effect on the fitted S_m or on the calculated single channel current i or conductance γ .

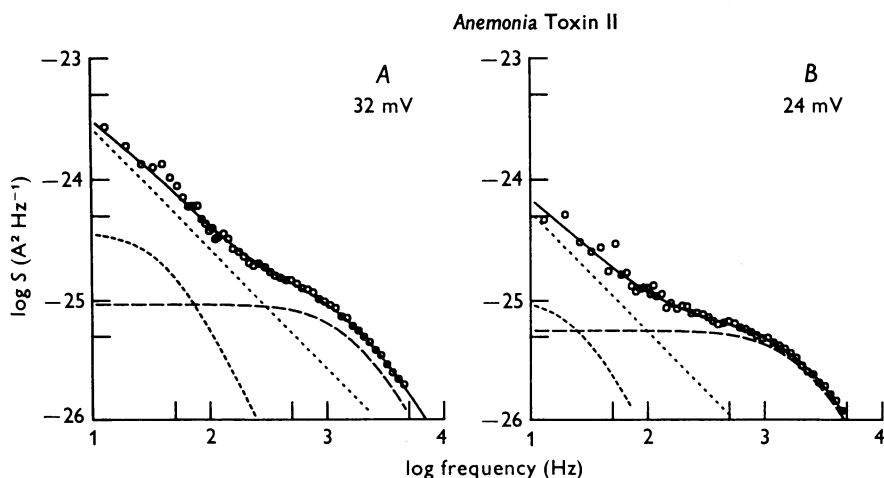


Fig. 3. Na current fluctuation spectra of a node treated with *Anemonia* Toxin II, 3×10^{-5} g/ml. (fibre 67). A few ml. toxin-containing test Ringer were perfused into pool A and the flow was stopped. Then, spectra were measured at 11–30 min after the toxin application. Finally, TTX Ringer was perfused and spectra were taken again at 43–61 min. Difference spectra for both solutions were plotted (\circ). The data were fitted according to eqn. (25) of Conti *et al.* (1976), as shown by the continuous line. The spectral components N/f , S_m and S_h are represented by dashed lines as marked in A.

TABLE 1. Conductance γ of a single Na channel (pS) estimated from fluctuation spectra at various pulse potentials and referred to $E = 0$ mV

Pulse potential (mV)	Normal†	Ni†	Iodate	<i>Leiurus</i>	<i>Anemonia</i>
8	4.9 (2)	1.3 (2)	3.7 (2)	8.2 (3)	—
16	6.5 (4)	3.6 (4)	5.7 (11)	6.4 (7)	4.3 (1)
24	10.0 (4)	4.5 (2)	5.8 (4)	6.9 (4)	7.7 (4)
32	9.4 (1)	6.3 (2)	4.3 (1)	3.1 (1)	7.6 (4)
40	11.9 (1)	—	—	—	4.2 (1)
48	—	5.3 (1)	—	—	—
Mean	7.9 (12)	4.0 (11)	5.4 (18)	6.7 (15)	7.0 (10)
S.E. of mean	0.9	0.6	0.4	0.5	0.6

The values given are averages with the number of observations indicated in parentheses.

† Values from Conti *et al.* (1976).

Average single-channel conductances obtained by fitting the power spectra are summarized in Table 1. The over-all averages for nodes treated with iodate, *Leiurus* venom, and *Anemonia* toxin are in the range 5.4–7.0 pS. Values for normal and Ni-treated fibres are included in the table for comparison. The most important point is that the single channel conductance is not changed much by agents that prevent sodium inactivation. Perhaps it is slightly reduced. In addition the apparent fall of γ at low potentials seen with normal and Ni-treated fibres is not so evident after iodate, *Leiurus*, or *Anemonia* treatment.

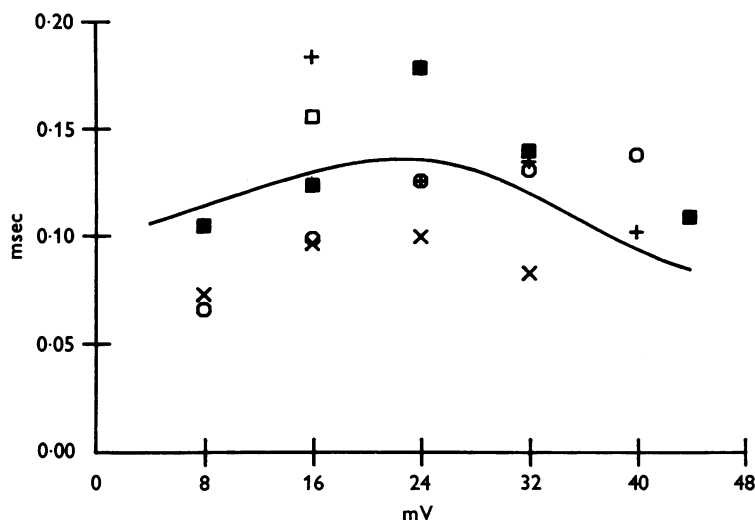


Fig. 4. Microscopic and macroscopic time constants τ_m . Mean τ_m values obtained from the Na current fluctuation spectra of normal or drug treated nodes are represented by the symbols: ○ normal, ■ first and □ last measurements during experiments with iodate, + *Leiurus* venom, × *Anemonia* Toxin II. The continuous line was drawn from eqns. (3)–(6) in Conti *et al.* (1976). These formulae were derived from a voltage clamp analysis of the macroscopic Na current of normal nodes at 13° C.

The average time constants τ_m obtained by fitting the power spectra are plotted together with the standard τ_m curve for our empirical description of voltage clamp currents in Fig. 4. As was the case with normal fibres, the macroscopic and microscopic measures of time constant agree within 50 %. In all iodate experiments, the first and last spectrum measured in test Ringer was at 16 mV, so an early point (■) and a late point (□) are given for τ_m at that potential. The indicated increase in the spectral τ_m at 16 mV from 124 to 156 msec seems to parallel the progressive increase of the macroscopic τ_m during iodate action.

The fitted $1/f$ noise expressed in terms of the quotient N/I^2 was

$(1.8 \pm 0.5) \times 10^{-4}$ for iodate treatment, $(1.5 \pm 0.3) \times 10^{-4}$ for *Leiurus* venom treatment, and $(2.1 \pm 0.6) \times 10^{-4}$ for *Anemonia* toxin treatment. These values are similar to that $(1.1 \pm 0.3) \times 10^{-4}$ found in normal fibres.

DISCUSSION

The most significant result of this paper is that Na channels of fibres with modified inactivation have high-frequency conductance fluctuations with a similar spectral distribution and yielding a similar single-channel conductance to normal fibres. The finding is consistent with the relative lack of effect of the same modifications on the rate of rise and the peak of Na currents during voltage clamp steps. Together the results argue that the inactivation mechanism operates in an all-or-nothing, open-close manner and is functionally separable from the activation mechanism. These conclusions are now considered in more detail.

All-or-nothing inactivation gate

So far we have emphasized that our conclusions are relatively independent of assumptions concerning the properties of the inactivation system. In the derivation of eqn. (17) in the previous paper the *h*-gating subunit was assumed to undergo first order transitions between single open and closed states. Because τ_m is much smaller than τ_h , the equation could be simplified and separated into the two components S_h and S_m . The component S_m alone sufficed to calculate γ , and the values of τ_h and h_∞ did not have to be known. The same conclusion was reached by considering the component of variance due to *m* fluctuations.

Several voltage clamp studies reviewed in Hille (1976) suggest that the inactivation system may have more than one closed state and more than one open state. Nevertheless if all open states have the same conductance and if all time constants are longer than τ_m then the separation of an S_m term is still possible as before and a voltage independent γ should be found. However, if opening of the *h*-gate is somehow graded rather than all-or-nothing, application of our S_m formula (eqn. (6)) should lead to a graded γ . Specifically γ would increase as *h*-gates open more. If anything our results show a small trend in the opposite direction. In normal fibres γ is 10.0, 9.4, and 11.9 pS at 24, 32, and 40 mV where h_∞ is 0.06, 0.016, and 0.005. In the drug-treated fibres of this paper the over-all average γ is 6.2 pS and the relevant h_∞ is unknown but certainly lies between 0.2 and 1.0. This is evidence that however many conformational states inactivation may have, the important conducting states have a similar conductance. In simple terms, the opening of *h*-gates is all-or-nothing. Thus both activation and inactivation mechanisms gate in a virtually all-or-nothing manner

with the reservation that activation gates might have at most a few percent imperfection in closing in normal fibres (Conti *et al.* 1976).

The inactivation gating subunit

Anemone and scorpion venoms are proteins with thirty-five to seventy amino acids, (Beress *et al.* 1975; Rochat, Rochat, Kupeyan, Miranda, Lissitzky & Edman, 1970), which presumably bind reversibly to some important part of the inactivation gating mechanism. For scorpion venoms this receptor is known to be accessible only from the outside (Narahashi, Shapiro, Deguchi, Scuka & Wang, 1972). That molecules as large as these venoms modify inactivation drastically with but small effects on fluctuations of *m*-gates (Fig. 4) and on the conductance of the open channel (Table 1) implies that the external receptor lies at some distance from both the activation mechanism and the conducting pore. Internal iodate and pronase also modify inactivation drastically again without much effect on activation or conductance (Stämpfli, 1974; Armstrong, Bezanilla & Rojas, 1973). Together these observations suggest that the inactivation mechanism resides in a large protein subunit which spans the membrane. This subunit is physically distinguishable from other components governing activation and forming the conducting pore.

The conductance of the Na channel

Our calculated γ values for Na channels may now be compared with those obtained by other methods and in other preparations (Table 2). The published γ values have been obtained through estimates of the density M of Na channels. Measurements of the binding or the rate of action of TTX yield the number of TTX receptors on the membrane. This number is usually equated with the number of Na channels. Measurements of displacement currents yield the maximum quantity of membrane charge movement which could participate in the activation of Na channels. Dividing this maximum charge by the minimum charge needed on physical grounds to activate one channel gives a maximum possible number of channels. Finally measurements of current fluctuations give a direct estimate of the number of channels M when eqns. (17) or (18) of the previous paper are used to interpret them.

We consider briefly some uncertainties arising in an interpretation of voltage fluctuations given by van den Berg *et al.* (1975) in the node of Ranvier. They measured noise attributed to h fluctuations on two myelinated nerve fibres of unspecified diameter from *Rana temporaria* at 20° C. The voltage noise was divided by $|Z_M|^2$ to convert it to equivalent current noise, where $|Z_M|$ was not measured but was assumed to be a fixed 40 M Ω . Also m , h , g_{Na} , and I were not measured but were assumed to be given by a model (Hille, 1971) for large fibres of *Rana pipiens*. It seems certain that errors could be introduced particularly by assuming a fixed value

of $|Z_M|^2$. In our calculations of phenomenological impedance given in the Methods of the previous paper, $|Z_M|^2$ was found to vary sixteenfold between 8 and 40 mV at 1 Hz. At frequencies where the roll-off of the S_h component of noise is expected, $|Z_M|^2$ also deviated strongly from its low-frequency value. Thus it seems necessary to use a voltage clamp in studying noise from the node of Ranvier to permit direct measurement of current noise and of the required kinetic and steady-state parameters without the ambiguities of a large number of assumptions.

TABLE 2. Summary of the conductance of single Na channels from estimates of Na channel density in the literature

Preparation	Criterion	γ (pS)	Reference	\bar{g}_{Na} used	Reference
Squid axon	TTX binding	2.2	Levinson & Meves, 1975	120 mS/cm ²	Hodgkin & Huxley, 1952
	Rate of TTX action	2-4	Keynes <i>et al.</i> 1975	120 mS/cm ²	Hodgkin & Huxley, 1952
	Gating current	> 2.5	Keynes & Rojas, 1974	120 mS/cm ²	Hodgkin & Huxley, 1952
	Current fluctuations	4.1	Conti, De Felice & Wanke, 1975	135 mS/cm ²	Conti <i>et al.</i> 1975
Frog muscle	TTX binding	8.6	Almers & Levinson, 1975	328* mS/cm ²	Hille & Campbell, 1976
Frog node	Gating current	> 2.9	Nonner <i>et al.</i> 1975	750 nS/node	Hille, 1971
	Voltage fluctuations	1-3	van den Berg <i>et al.</i> 1975	750 nS/node	Hille, 1971
	Current fluctuations	6.6†	This paper	—	—

All except last γ value are obtained by dividing \bar{g}_{Na} of the preparation by the number of Na channels.

* Almers & Levinson used \bar{g}_{Na} of 22-53 mS/cm² and hence estimated γ values close to 1 pS.

† Average of all our observations in Table 1 except those on Ni-treated fibres. Calculated from fluctuations without the need to assume a value for \bar{g}_{Na} .

Table 2 summarizes estimates of γ which are obtained by dividing the listed values of limiting sodium conductance \bar{g}_{Na} by the M values determined as described. In the two calculations from gating charge movement, the result is a lower limit. The estimated γ values for squid giant axons lie in the narrow range 2-4.1 pS. The value from current fluctuations in the squid was obtained from m -fluctuations at 4° C via eqn. (20) of the previous paper and using average values of m , h , and I obtained in separate experiments on the same kind of squid. The estimated γ values for frog nerve and muscle lie in the wider range 1-8.6 pS. The low 1-3 pS value from voltage fluctuations (van den Berg *et al.* 1975) is subject to the uncertainties already discussed. The 8.6 pS value from TTX binding to frog muscle is actually much higher than the 1 pS value originally estimated by Almers & Levinson (1975) because we have used a higher value for

\bar{g}_{Na} which we believe to be justified by newer voltage clamp work. In any case, all the γ values in Table 2 including ours are reasonably similar, lending some confidence in the variety of methods used and in the conclusions. These newer values are much smaller than the 100–440 pS values estimated earlier from indirect arguments (Hille, 1970) and also suggest that despite the higher Na content of sea water, the Na channel of squid axons may not be more conductive than that of frog nerve.

With an estimate of the channel conductance we can calculate the number and surface density of Na channels. Our \bar{P}_{Na} of $4.3 \times 10^{-9} \text{ cm}^3 \text{ sec}^{-1}$ given in the previous paper for a $15 \mu\text{m}$ fibre is equivalent to a \bar{g}_{Na} at $E = 0 \text{ mV}$ of 683 nS per node under our conditions. If γ were 6.8 pS then there would be 10^5 Na channels per node. The same result is obtained by dividing the corresponding single channel permeability $4.3 \times 10^{-14} \text{ cm}^3 \text{ sec}^{-1}$ into \bar{P}_{Na} . At the resting potential about twenty-five of these channels would be open on the average and each open channel would be passing about 1.3 pA of current or 8×10^6 ions per second. Such high turn-over rates are several orders of magnitude higher than the turn-over rates for carriers or ordinary enzymes at 13°C and constitute the strongest argument that Na channels are pores. The membrane area of a node of Ranvier is only poorly known, but assuming $50 \mu\text{m}^2$ gives a mean channel density of $2000 \mu\text{m}^{-2}$ and a mean separation of 225 Å between channels.

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